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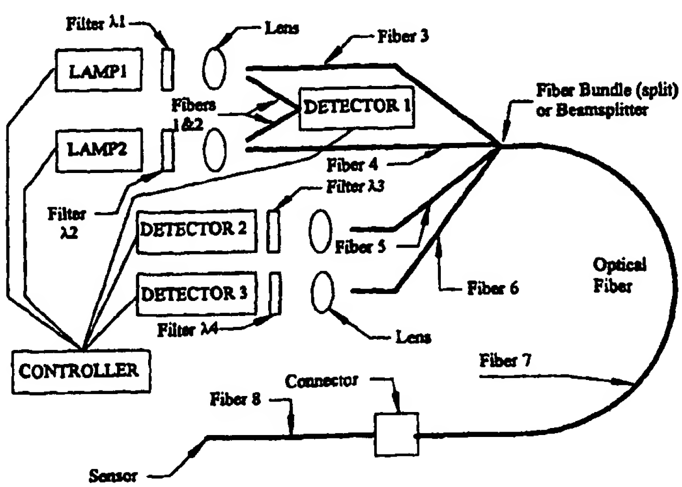

(57) Abstract

Optical sensors are used to measure the concentration of oxygen or other analytes in a sample. The radiometric method utilizes an indicator dye which is pyranthrene, flavanthrene or a suitable derivative dispersed or immobilized in an analyte permeable matrix. The matrix is excited with light at one or more wavelengths, or fluorescence emissions of the indicator are measured at one or more wavelengths, or both excitation and emission are performed and measured at multiple wavelengths. These indicator dyes exhibit different sensitivity to quenching by the analyte at different excitation wavelengths and also at different emission wavelengths. Each emission resulting from a different excitation is divided by the measured intensity of its excitation to compensate for illumination variations. The ratio of the resulting signals for each excitation is then used for determining the analyte concentration. Alternatively, the ratio of emissions at a single excitation wavelength are used in a similar manner.

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METHOD AND MATERIAL FOR RATIOMETRIC FLUORESCENT DETERMINATION OF ANALYTE CONCENTRATION

BACKGROUND OF THE INVENTION

1. Field of the Invention:

The present invention relates to optical chemical sensors, and more particularly to an improved optical sensor system for use in the measurement of analytes such as oxygen, sulfur dioxide, nitric oxide, the anesthetic agent HALOTHANE, halogens, and other materials known to quench the fluorescence of polycyclic aromatic hydrocarbons (PAHs) and polycyclic heterocyclic aromatic compounds (HACs). These measurements are used to determine the concentration of analytes in gaseous and fluid mixtures. These measurements are also used for determining pressure, glucose concentration, and various other parameters by extension.

1. Brief Description of the Prior Art:

The use of fiber optic sensor systems for the measurement of parameters such as partial pressure of oxygen (PO2) and of concentration or pressure of other analytes through the use of a luminescent material is well known. These sensors have been used in medical applications (both invasive and non-invasive), chemical, biochemical, environmental, military, and industrial analysis. Immunity to electrical interference, extended calibration intervals, and small size have made them particularly important to the medical community.

The principle utilized in these devices for measuring concentration of analytes, such as oxygen, is that fluorescence emissions from certain dyes is quenched by the analyte in accordance with the Stern-Volmer relationship. Thus a fluorescent dye whose fluorescence is quenched by the analyte is incorporated in the sensing device as indicator. In medical applications

systems incorporating these sensors are often part of multipurpose catheters which measure blood pressure, provide for fluid infusion and sample withdrawal, and may include additional sensors or therapeutic tools.

Optical oxygen sensors may also be used for extracorporeal monitoring during heart/lung bypass procedures, dialysis, or other blood treatment procedures, for arterial line monitoring, both *ex-vivo* and *in-vivo*, tissue monitoring of peripheral perfusion, augmentation of cardiac catheters, and many others. Non-medical uses include analysis in food and beverage manufacturing, water quality monitoring, chemical and biochemical process control, environmental monitoring, and the like.

Optical chemical sensors are typically fabricated by mounting the indicator dye to the end of an optical fiber, window, or rod. Light is transmitted down the optical element from an excitation source, such as a laser, lamp, or an LED (light emitting diode). At the tip of the fiber, window or rod the analyte being sensed interacts with the indicator dye to change its optical properties. It is this change which is measured by measuring the light emitted by the fluorescent indicator dye. Various methods are used to separate the fluorescent emission light returning from the indicator dye from excitation light scattered within the system. For example optical filters, beam splitters, bifurcated fiber bundles, or like devices are often used to separate excitation and emission light and to measure the emission light. The results of the measurement may be displayed in any number of ways such as a digital display, and the like. The thin indicator dye matrix layer used in most optical sensors results in a relatively fast response time.

In an alternative configuration, the indicator dye is excited by the application of an electric field instead of light. This approach is generally referred to as electroluminescence.

Thus, the optical oxygen sensor is based on the principle of quenching.

There are numerous fluorescent compounds that exhibit a predictable change or quenching of fluorescence when exposed to oxygen or other analytes and therefore can be used as indicator dyes in the above-summarized optical chemical sensors.

However, due to the nature of the dyes used in the prior art and to other factors, several problems exist with prior art fiber optic chemical sensors. Some sensors are not stable. Performance varies significantly over time. Other sensors require expensive light sources or sophisticated computers.

The prior art has strived to eliminate some of the foregoing problems and, as a result, provided optical chemical sensors having improved performance and stability. These generally incorporate means for referencing the analyte signal to a stable independent reference signal. Optimum performance is obtained when the analyte signal and a reference signal occur within the spectrum of a single indicator dye.

Methods used to obtain this independent reference signal have included providing a second sensor not exposed to the analyte, the use of a separate indicator compound, the use of different chemical forms of an indicator, the use of fluorescence lifetime measurements, and the reported use of emission ratios. Even these improved prior art systems have significant drawbacks. Some of the drawbacks are low fluorescence intensity, variations in excitation light intensity, transmission changes in the optical path, concentration variations of the indicator dye(s), leaching of the dye out of the sensor, changes in the local environment at the sensor matrix such as physical damage or conformational variations, limited dye stability, power intensive light source needs, photodegradation of the dye(s) differential photodegradation of multiple dyes, complex apparatus requirements, and inadequate reference compensation. Lifetime measurement drawbacks also include the need for very high speed data collection and analysis.

It is also desirable to have dyes which can be excited at wavelengths above 400 namometer (>400nm excitation) because these dyes can be used with inexpensive LED's instead of power intensive and costly lamps, lasers, or flash lamps.

From among the numerous prior art patent and other references which describe the above-summarized and related features of optical chemical sensors the following are noted.

U.S. Patent No. 5,094,959 describes an oxygen sensor using ratiometric emission measurements with single excitation at near ultraviolet wavelength.

U.S Patent No. 4,476,870 describes a basic fiber optic oxygen probe.

U.S. Patents Nos. 5,151,603, EP,0,442,060,A2, and 5,462,880 describe ratiometric oxygen measurement systems using 2 dyes mixed together.

U.S. Patent No. 4,849,172 discloses a method for increasing the solubility of a polycyclic aromatic hydrocarbon in a polysiloxane polymer.

U.S. Patents Nos. 4,712,865, 5,015,715, and 4,746,751 disclose methods for immobilizing a polycyclic aromatic hydrocarbon on a polysiloxane polymer.

U.S. Patent No. 5,681,532 describes a fluorocarbon based oxygen permeable matrix for use in optical oxygen sensors along with a 2 dye system. An article in Anal. Chem. 1985, **57**, pp 2556-2561, (author O. S. Wolfbeis) and U.S. Patent No. 5,155,046 describe the effect of HALOTHANE on PAH indicators.

The monograph "Photophysics of Aromatic Molecules", (*John B. Birks*, 1969 page 444 & 504 describes that the oxygen sensor technology can also be used to measure nitric oxide, carbon tetrabromide, carbon tetrachloride, ethyl iodide and many other halogenated compounds.

As described in SPIE Vol. 990 1988, 116-120 the oxygen sensor technology can also be used to measure sulfur dioxide, pressure as described

in U.S. Patent 5,359,887, and glucose as described in U.S. Patent 5,034,189.

Most of the patents listed above and U.S. Patents No. 4,917,491 utilize the fluorescent dye in a fiber optic sensor arrangement. U.S. Patent No. 5,629,533 utilizes the fluorescent dye in an electroluminescent analyte sensor arrangement.

Another pertinent background to the present invention relates to the polycyclic aromatic hydrocarbon pyranthrene. The following four scientific publications are of interest:

"Solvent effect on $^1O_2$ yield and the Mechanism of Polycylic Hydrocarbon Triplet State Quenching by Oxygen", A.P Darmanyan, Chemical Physics Letters, 1983, Vol. 96, 3, 383-9. This paper describes singlet oxygen production as a tool to study the effect of solvent on the interactions of triplet states in certain PAH's including Pyranthrene.

"Spectroscopic Investigation of Fluorescence Quenching Agents. Part IV.", J. C. Fetzer, Applied Spectroscopy, 1993, Vol. 47, 11, 1775-9. This paper describes the selective quenching of certain PAH's, including Pyranthrene, by Nitromethane as a means of classification.

"Spectroscopic Investigation of Fluorescence Quenching Agents. Part II", J. C. Fetzer, Applied Spectroscopy, 1992, Vol. 46, 8, 1260-5. This paper describes the selective quenching of certain PAH's, including Pyranthrene, by Nitromethane as a means of classification.

"The Triplet Energies of Azulene, b-Carotene, and Ferrocene", W.G. Herkstroeter, Journal of A.C.S., 1975, Vol. 97, 15, 4161-7. This paper describes the triplet energies of Azulene, b-Carotene, and Ferrocene by investigating their interaction with the triplet states of various PAHs including Pyranthrene.

Because of the problems that still prevail in the state-of-the-art of optical chemical sensors, need for improvement regarding several features of

these devices still exists. Specifically, a need exists to improve the stability of the indicator dye and matrices in order to improve their overall performance. A need also exists for a dye with a large extinction coefficient to obtain increased fluorescence intensity. A need also exists for longer wavelength dyes compatible with LED's. A need also exists for an improved dye with self referencing dual wavelength properties, both for excitation and emission.

In light of the foregoing, the principal objective of the present invention is to provide an improved optical chemical sensor system which overcomes the above-described shortcomings of the prior art and fulfills the above-listed needs.

SUMMARY OF THE INVENTION

The present invention provides a method and apparatus by which a single fluorescent dye is used both as the analyte indicator and as a reference element in an optical chemical sensor for sensing analytes such as oxygen, sulfur dioxide, nitric oxide, and many halogenated compounds which are known to quench the fluorescence of polycyclic aromatic hydrocarbons. In accordance with the invention the polycyclic aromatic hydrocarbon pyranthrene or the polycyclic heterocyclic aromatic compound flavanthrene, or a suitable derivative of these compounds is used as the single reference dye of the optical chemical sensor. This is possible because these dyes exhibit significantly different analyte quenching slopes at different excitation wavelengths, and also significantly different analyte quenching slopes at different emission wavelengths. The excitation wavelength of these dyes is advantageously in the range of the light generated by light emitting diodes and therefore the sensors utilizing these dyes can operate with LEDs. Advantageously these dyes also exhibit large Stokes shifts of emission wavelengths, and large extinction coefficients which result in strong fluorescence emissions. Excitation of one of these dyes at two or more

wavelengths, coordinated with the measurement of both the excitation intensities and one or more emission intensities, provides a self referencing system. Alternatively, excitation of these dyes at a single wavelength while measuring two or more emission intensities also provides a self referencing system. In combination with a suitable gas permeable matrix, such as polydimethylsiloxane, pyranthrene, flavanthrene or a suitable derivative of either of these two dyes provides improved means for the measurement and normalization of measurements of the analytes known to quench the fluorescence of polycyclic aromatic hydrocarbons.

In accordance with this invention, a hydrophobic oxygen (or other analyte) sensitive matrix is formed incorporating the pyranthrene, flavanthrene or suitable derivative of either of these two dyes. A suitable derivative is one that is sensitive to changes in oxygen (or other analyte) concentration in substantially the same manner as the parent compound, pyranthrene or flavanthrene. A preferred gas permeable matrix is polysiloxane polymer. The fluorescent pyranthrene, flavanthrene or suitable derivative dye dispersed or immobilized in the siloxane polymer matrix is coated at the end of a fiber, cast into films, or mounted with any of the other common methods for preparing optical sensors. For measurement the matrix with the dye is exposed to the analyte. The sensor apparatus in accordance with the invention further includes light sources, preferably and advantageously in accordance with the present invention one or two LEDs, preferably means for measuring the excitation light at one or two wavelengths, means for conducting the excitation light at one or two excitation wavelengths bands to the sensor dye, means for separating the fluorescent emission light at one or two wavelengths bands from the excitation light, means for measuring the emitted fluorescent light at one or two wavelengths bands, and means for calculating ratios of the measured intensities to arrive to the concentration of the analyte in a sample,

based on the spectral response curves of the pyranthrene, flavanthrene or derivative dye which is used in the sensor.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1 is a diagrammatic view of a first preferred embodiment of an optical chemical sensor apparatus in accordance with this invention;

Figure 2 is a diagrammatic view of a second preferred embodiment of an optical chemical sensor apparatus in accordance with this invention;

Figure 3 is an expanded view in section of an optical fiber optical chemical sensor in accordance with this invention;

Figure 4 is a diagrammatic illustration of a test run using a sensor of this invention;

Figures 5, 6, 7 and **8** are diagrammatic illustrations of the spectral response of a pyranthrene test cell;

Figure 9 discloses the chemical structure of flavanthrene;

Figure 10 discloses the chemical structure of pyranthrene;

Figure 11 is a diagrammatic illustration of the spectral response of flavanthrene dye.

DETAILED DESCRIPTION OF THE INVENTION

The following specification taken in conjunction with the drawings sets forth the preferred embodiments of the present invention. The embodiments of the invention disclosed herein are the best modes contemplated by the inventors for carrying out their invention in a commercial environment, although it should be understood that various modifications can be accomplished within the parameters of the present invention.

The present invention is described below in connection with measuring oxygen concentration in an analyte fluid, because measuring oxygen, primarily in medical applications, is one or the most frequent use of optical chemical sensors, and the presently preferred embodiments of the invention are also

primarily directed to this use. It should be clearly understood however, that instead of oxygen other analytes of the type which quench the fluorescence of PAH dyes can also be measured in accordance with the present invention.

As is known in the prior art, in an optical chemical sensing apparatus for measuring partial pressure of oxygen (P_{O2}), a polycyclic aromatic hydrocarbon or polycyclic heterocyclic aromatic compound (PAH or HAC) is immobilized or dispersed in a gas permeable polymer such as polysiloxane. The PAH or HAC has an emission intensity which is inversely proportional to the oxygen concentration in accordance with the Stern-Volmer relationship. This relationship is described by equation **(1)**:

$$I_O/I = 1 + k_q t_O[Q] \qquad \textbf{(1)}$$

where I is the intensity, I_O is the intensity without quenching, k_q is the rate constant of the quenching reaction, t_O is the life of the emission in the absence of quencher, and [Q] is the concentration of quencher. t_O and k_q are constants which vary widely among PAH's. I_O is a constant which must be calibrated for a given optical system with sensor. In practical actual instruments in accordance with the state-of-the-art, equation **(1)** is typically further refined to accommodate certain offsets in the system, temperature coefficients for the various constants, and variations in the source light intensity as measured by a separate photodetector.

For the dyes of this invention where two or more wavelengths exhibit differing slopes, the equations become:

$$I_{Oa}/I_a = 1 + k_{qa} t_{Oa}[Q] \qquad \textbf{(2)}$$

$$I_{Ob}/I_b = 1 + k_{qb} t_{Ob}[Q] \qquad \textbf{(3)}$$

Where "a" and "b" in subscripts designate the values of "I_O", "I", "q" and "t" for the respective wavelength "a" and "b". In general, I_{Oa} / I_{Ob} is a constant for any given optical system independent of the sensor attached. These equations can then be solved for any random sensor attached to the optical system as

follows:

$$I' = I_{Oa}/I_{Ob} \quad \textbf{(4), and}$$

$$[Q] = ((I_a / I_b) \cdot I') / ((k_{qa} t_{Oa}) + (k_{qb} t_{Ob}) \cdot (I_a / I_b)) \quad \textbf{(5)}$$

Thus, in an optimum configuration the present invention, by utilizing pyranthrene or flavanthrene (or a suitable derivative of either of the two) enables the fabrication and use of a factory calibrated optical system which can be used interchangeably with any sensor without field calibration. The prior art has attempted to obtain this result through the use of two different dyes, but only with limited success.

As is described in more detail below, the pyranthrene or flavanthrene (or suitable derivative) dye can be, and preferably is covalently bonded to a siloxane polymer to provide a hydrophobic matrix from which the dye is not leachable.

The PAH and HAC dyes used in the prior art typically absorb light in the ultraviolet region of the spectra and emit at longer wavelengths, typically in the blue or green region of the spectrum. The pyranthrene or flavanthrene (or suitable derivative) dyes of this invention are among a very limited number of stable PAH's which excite in the blue region and have emission in the green region of the spectrum.

Whereas the fluorescence of certain PAHs and their dynamic quenching by oxygen and other analytes is well known, the present inventors are unaware of any prior description of fluorescence by flavanthrene, and are aware of only limited scientific literature (four articles) describing the fluorescent properties of pyranthrene. These are listed in the Brief Description of the Prior Art section of the present application for patent. None of the prior art pertaining to pyranthrene envisions this invention, which is based on the discovery of unusual quenching properties of pyranthrene and flavanthrene. Whereas other

PAHs and HACs tested show substantially uniform quenching across the entire excitation and emission spectrums, these indicators demonstrate substantially different quenching constants for different wavelengths in the excitation and emission spectra.

Thus, in one embodiment or preferred mode of the method and apparatus of the present invention two or more excitation wavelengths and a common emission wavelength are measured to provide a ratiometric intensity measurement. In this manner, by providing self-reference, the invention improves performance, increases stability, minimizes interference from environmental perturbations and optical system variations, and the like.

In another embodiment or preferred mode of the method and apparatus of the present invention a single excitation wavelength and two or more emission wavelengths are measured to provide a ratiometric intensity measurement, again providing a self-reference that improves performance, increases stability, minimizes interference from environmental perturbations and optical system variations, and the like.

In still another embodiment or preferred mode of the method and apparatus of the present invention a combination of multiple excitation and multiple emission wavelengths are measured to provide cross-referenced ratiometric intensity measurements, providing multiple self reference that improves performance, stability, and minimizes interferences. Of course it will be readily understood by those skilled in the art that reference to a "wavelength" in this description refers to a central wavelength and a band width corresponding thereto, which is often but not necessarily is characterized by its full width at half maximum intensity. The term "wavelength" thus refers to a band in this specification and claims, unless another meaning is apparent from context.

It has been discovered as still another unique feature of the present

invention that the pyranthrene and flavanthrene dyes have longer excitation wavelengths than most previously disclosed PAHs and HACs for use in chemical sensors. This makes it possible to use relatively inexpensive LEDs as sources of excitation light in the light sensing apparatus incorporating these dyes as sensors. A further uniqueness of this invention is the discovery that these dyes have a large extinction coefficient and strong fluorescence intensity.

A still further unique feature of the present invention is the discovery that the pyranthrene and flavanthrene dyes have a steeper oxygen response slope than most previously disclosed PAHs and HACs for use in similar optical chemical sensors. This makes the sensing devices incorporating these dyes more sensitive and therefore more accurate.

To form the actual sensor, the pyranthrene or flavanthrene dye or a suitable derivative of either one of these two dyes can be perfused in an insoluble matrix. However, it is preferred to immobilize the dye by covalently bonding it to the insoluble matrix, which is preferably a polysiloxane matrix. Alternatively, the pyranthrene or flavanthrene dye (or suitable derivative) can be perfused or immobilized in a fluoropolyurethane matrix, as is done with other agents in U. S. Patent No. 5,681,532, incorporated herein by reference. Generally speaking, chemical processes and methods normally used in the art for covalently bonding PAH compounds to a polysiloxane matrix can be utilized for bonding the pyranthrene or flavanthrene dye (or suitable derivative) to a polysiloxane matrix. For example, the methods of coupling described in U. S. Patent Nos. 4,746,751 and 5,015,715 can be used. U. S. Patent Nos. 4,746,751 and 5,015,715 are incorporated herein by reference. The methods and compositions utilized in the state-of-the-art for bonding PAH compounds to a polysiloxane matrix usually involve the use of linking moieties that covalantly link the PAH to the matrix through several atoms. A

"suitable derivative" of pyranthrene or flavanthrene within the scope of this invention is a chemically substituted pyranthrene or flavanthrene molecule which substantially retains one or more of the important fluorescent and quenching properties of the parent compound, pyranthrene or flavanthrene, respectively. In fact, a pyranthrene or flavanthrene moiety that is covalently linked to a matrix can also be viewed as a "pyranthrene or flavanthrene derivative", because any linking moiety that is attached to the pyranthrene or flavanthrene itself can be viewed as a substituent. As experience in the prior art with other PAH compounds used as optical sensors has demonstrated, the linking moieties that covalently bond PAHs to the matrix can be selected to avoid adverse effects on the fluorescent and quenching properties of the PAH compound.

A presently preferred method of using pyranthrene in an optical sensor is to link it to a polydimethylsiloxane methyl vinyl copolymer, which is thereafter cross-linked with polymethylhydrosiloxane as described below.

Example 1: Preparation of Optical Chemical Sensor Matrix Having Covalently Linked Pyranthrene

The chemical pyranthrene was obtained from (NBS Biologicals Ltd., Huntingdon, U. K.). It can also be prepared in accordance with the chemical literature, see *E. Clar* Berichte Deutscher Chemischer Gesellschaft, **76**, 332 (1943). 5×10^{-4} mole pyranthrene and 5 grams of polydimethylsiloxane methyl vinyl copolymer PS925 (United Chemical Tech., Bristol Pa.) was measured into a 20 ml vial equipped with a stirrer. The solution was stirred and heated under nitrogen. Heating was continued until the temperature of the silicone reached 180 ° C, at which time 0.5 g of $AlCl_3$ (Aldrich Chemical Co.) was added and the mixture was heated for an additional 15 minutes. The mixture was then cooled to room temperature and the $AlCl_3$ was quenched with water. The organic phase was extracted with 50 ml of xylenes (Aldrich

Chemical Co.), washed twice with water, then dried over magnesium sulfate (Aldrich Chemical Co.). The xylenes were evaporated and the polysiloxane containing the covalently bonded pyranthrene was filtered through a glass wool plug. The dye modified PS925 polysiloxane was mixed 1:1 with polymethylhydrosiloxane PS119 (United Chemical Tech.) along with a trace of PC075 catalyst platinum, 1,3-diethenyl-1,1,3,3-tetramethyldisiloxane complexes in polydimethlysiloxane, vinyl terminated (United Chemical Tech.). One drop was applied to a test cell then cured at 55 ° C for 2 hours, resulting in a thin film ready for testing and use.

Example 2: Preparation of Flavanthrene

Flavanthrene was prepared from flavanthrone (TCI America, Portland Oregon) by heating a mixture of flavanthrone (0.57 g) and 20.0 ml of 57% HI (Aldrich) to reflux for 20 hours. The hot solution was poured into 300 ml of 2% aqueous sodium bisulfite solution (Sigma Chemical). The resulting precipitate was filtered, washed with cold water and air-dried. Further purification was carried out by adding the crude solid product to xylenes (Aldrich). Insoluble impurities were removed by filtration The xylenes were removed by evaporation at 65 °C under reduced pressure. A dilute solution of approximately 0.01 mmol was prepared in toluene and placed in a 1 cm square test cell for testing.

The excitation (EX) and emission (EM) spectra of the pyranthrene polysiloxane matrix and of the flavanthrene materials prepared above, were scanned with a Perkin-Elmer LS-5 spectrofluorimeter in the presence of oxygen (100% O_2), room air (21% O_2), and Nitrogen (0% O_2). The intensity measurements are set forth in **Table 1**.

TABLE 1

PAH	EX	EM	Oxygen	Room Air	Nitrogen	I_{N2}/I_{air}	Scan #
Pyranthrene	410	441	28.9	60.6	122.9	2.03	a
Pyranthrene	410	500	27.2	47.5	79.3	1.67	b
Pyranthrene	456	500	74.8	144	204.3	1.42	c
Flavanthrene	410	444	62.4	120.1	198.4	1.65	d
Flavanthrene	410	472	106.9	209.3	299.2	1.43	e
		Scan #		Slope differential (%)			
		a vs. b		21.6			
		a vs. c		43			
		b vs. c		17.6			
		d vs. e		15.3			

Where Slope differential (a vs. b)= $100*((I_{N2}/I_{air})_a - (I_{N2}/I_{air})_b)/(I_{N2}/I_{air})_b$ and where I_{N2} and I_{air} represent measured light intensities in the presence of nitrogen and air respectively, and the subscripts "a" and "b" refer to the measurements obtained in the corresponding scan designated "a" and "b".

Among all PAH compounds tested in connection with the present invention only pyranthrene and flavanthrene exhibited significant (>10%) dual wavelength variation in fluorescence (singlet) quenching coefficient. It should be readily apparent to those skilled in the art based on the foregoing data that the use of the single pyranthrene (or flavanthrene) indicator system is superior to the mixing of two different dyes. The superiority is due to the elimination of differential photodegradation, differential temperature coefficients,

differential chemical interferences, and the like.

Detailed Description of the Preferred Embodiments of the Optical Sensing Apparatus of the Invention

Referring to the drawings which illustrate the preferred embodiments of the apparatus used in accordance with the invention, **Figure 1** illustrates a preferred form of the spectrofluorimetric system **10** for measurement of analyte in a continuous flow environment, or of samples of only a few microliters. The system includes a control section (CONTROLLER) for operation of multiple light sources (LAMP1) and (LAMP2), reading of the signals from photodetectors (PHOTODETECTOR 1, 2, 3), computations, and display handling. The CONTROLLER may be connected to a general purpose computer or may itself include a processing unit, and receives as input the data of measurements. The processing unit or computer calculates analyte concentration by using an algorithm that is based on the equations described above and includes as constants the previously observed characteristics of the indicator dye's response to the analyte at two excitation or two emission wavelengths or both.

The light sources may be in the form of LEDs, incandescent bulbs, lamps, or strobe lights. Light from each source (LAMP1, LAMP2) in sequence passes through a corresponding optical filter ($\lambda 1$, $\lambda 2$) for excitation wavelength and bandwidth selection, then enters two corresponding fibers (fibers 1& 3, or fibers 2 & 4). Fibers 1&2 feed source light into Detector 1 where the intensity of each lamp is measured in order to compensate for any variations. Fibers 3 and 4 feed source light into the multifiber bundle labeled fiber 7.

The distal end of fiber 7 joins with an optical analyte sensor fiber 8 through an optical fiber connector (CONNECTOR). The source light reaches

the sensor tip (SENSOR) where it excites emission light from the indicator dye that is in accordance with the present invention, that is pyranthrene or flavanthrene or a suitable derivative, more preferably pyranthrene covalently linked to cross-linked polydimethylsiloxane methyl vinyl copolymer. The configuration of the sensor tip is shown in detail in Figure 3.

Fluorescent emission light returning from the optical analyte sensor material passes through fiber 8, back through the connector, and then into fiber 7. The emission light is then split with a portion entering fibers 5 and 6. This emission light emerges from the proximal end of fibers 5 and 6, is focused with a lens, optically filtered for specific wavelengths by filters 13 and 14, then illuminates detectors 2 and 3 respectively. The electronic output of detectors 1, 2 and 3 is connected to the controller for processing. In this embodiment the optical cable also acts as a beam splitter, although in other embodiments other devices may be used for this purpose. Photodetectors 2 and 3 may also be replaced with a suitable spectrometer for measurement of the entire emission spectrum.

Data processed by the control unit may be displayed digitally or used as the input to another computer system. It should be understood that the system of **Figure 1** is for purposes of illustration only and that other processing and light control and transmission and receiving systems may be used.

Figure 2 is a diagram of an alternative preferred embodiment used in preliminary optical sensor tests. The system includes a control section (CONTROLLER) for operation of multiple light sources (LED $\lambda 1$) and (LED $\lambda 2$), reading of the signals from Detectors 1 & 2, computations, and display handling. LED $\lambda 1$ (430nm) and LED $\lambda 2$ (470nm) are alternatively illuminated to provide source light. This source light passes through a common excitation filter 14 where longer wavelengths are removed (470nm short pass) and into a glass optical window. Attached to the optical window with an index matching

optical fluid or adhesive is a clear plastic substrate with indicator dye sensor film, containing the pyranthrene or flavanthrene dye in accordance with the invention, preferably pyranthrene covalently linked to cross-linked polydimethylsiloxane methyl vinyl copolymer. The source light passes from the optical window through the substrate and into the sensor film.

A portion of the emission light resulting from indicator dye excitation by the source light passes back through the substrate, through the optical window, and then through emission cut-off filter λ3. The emission filter (480nm long pass) removes any scattered excitation light from the returning light and allows the remainder to pass into Detector 2 for measurement. This embodiment represents a disposable sensor element without the requirement for a costly fiber optic connector. Simple coated films may be used.

Figure 3 illustrates the end of an optical chemical sensor structure 120 in accordance with this invention. In this embodiment the front face 122 of the optical fiber 128 is coated with the indicator matrix 125 using the immobilized pyranthrene materials previously described, the analyte sensor then being overcoated with an opaque analyte permeable layer 127 (Black RTV silicone GE 283).

The physical structure of the optical chemical sensors in accordance with this invention may vary widely because of the ease of use, stability, and relatively high fluorescence intensity. The diameter of the optical fiber in a sensor may vary from a few microns to 1/2 inch or more. One of the advantages of the present invention is the ability to provide an optical chemical sensor in which the diameter is quite small and easily inserted invasively in a hypodermic syringe or other form of invasive catheter. In the case of coating(s) on the end face of the optical fiber, films in the range of 20 to 40 microns are preferred, although films greater than 300 microns have also been found to be effective with somewhat slower response.

The optical fiber may be of plastic material or of glass. The term optical fiber as used in this invention also includes optical windows and rods which may be used in sensors without cladding.

Figure 4 charts the oxygen response of the signal ratio for a sensor fabricated in accordance with this invention using pyranthrene immobilized in silicone.

Figure 5 illustrates the fluorescent emission response of a pyranthrene polysiloxane polymer at 500 nm when excited in a range of excitation wavelengths between 260 and 490 nm in the presence of varying concentrations of oxygen. Thus, by way of example and not a limitation on this invention, curve 65 represents the excitation spectrum of the sensor material at 0% oxygen. Curve 65a represents the response at 20.3% oxygen, and curve 65b represents the response at 100% oxygen.

Figure 6 charts the emission ratios at 500 nm when the excitation is at 412nm and at 457nm in the presence of varying concentrations of oxygen. This illustrates one computational method for self compensation in the measurement of oxygen.

Figure 7 illustrates the emission spectral response of a pyranthrene sensor when excited at 410nm. Thus, by way of example and not a limitation on this invention, curve 67 represents the excitation spectrum of the sensor material at 0% oxygen. Curve 67a represents the response at 20.3% oxygen, and curve 67b represents the response at 100% oxygen.

Figure 8 charts the ratio of the 442nm and 470nm emissions, when excited at 412 nm in the presence of varying concentrations of oxygen. This illustrates another computational method for self compensation in the measurement of oxygen.

Figure 9 discloses the chemical structure of the flavanthrene molecule.

Figure 10 discloses the chemical structure of the pyranthrene molecule.

Figure 11 illustrates the emission spectral response of flavanthrene in toluene when excited at 410nm in the presence of varying concentrations of oxygen. Thus, by way of example and not a limitation on this invention, curve 69 represents the excitation spectrum of the sensor material at 0% oxygen. Curve 69a represents the response at 20.3% oxygen, and curve 69b represents the response at 100% oxygen.

Optical chemical sensor systems or apparatus can be constructed in accordance with the present invention to measure concentrations of oxygen, nitric oxide, sulfur dioxide, or halogens, or other analytes known to quench the fluorescence of polycyclic aromatic hydrocarbons. Sensors constructed in accordance with the present invention can also be used for measuring glucose by means of an enzymatically coupled system, and aerodynamic pressure by means of an oxygen concentration coupled system. Moreover, optical chemical sensor systems or apparatus can also be constructed in accordance with the present invention where the initial excitation is provided by applying an electric field to the probe containing the dispersed or immobilized pyranthrane, flavanthrane (or suitable derivative) dye.

What has been described above is an improved self-referencing optical chemical sensor system which overcomes several deficiencies of the prior art systems. It should be understood that while several embodiments of the invention have been shown and described, modifications may be become readily apparent to those skilled in the art in light of the foregoing disclosure. Therefore, the scope of the present invention should be interpreted solely from the following claims, as such claims are read in light of the disclosure.

WHAT IS CLAIMED IS:

1. An optical chemical sensor system for determining the concentration of an analyte, comprising:

a probe having a polymeric matrix and an optical sensing compound dispersed or immobilized in the matrix, the sensing compound being responsive to excitation by light and providing a fluorescent emission of light in response to the excitation, said fluorescence emission being quenched by the analyte in a relationship that is a function of the concentration of the analyte, the sensing compound being selected from the group consisting of pyranthrene, a derivative of pyranthrene, flavanthrene and a derivative of flavanthrene;

a source of light that provides excitation to the optical sensing compound in the probe;

a detector that measures the intensity of the fluorescent emission of the sensing compound in the probe, and

means utilizing an algorithm for calculating the concentration of the analyte from the measured intensity of the fluorescent emission, said algorithm being based on the spectral response curve of the optical sensing compound.

2. The optical chemical sensor system in accordance with Claim 1, where the source of light provides excitation at at least one predetermined wavelength and where the detector measures the intensity of the fluorescent emission of the sensing compound in the probe at at least two wavelengths of fluorescence emission.

3. The optical chemical sensor system in accordance with Claim 1, where the source of light provides excitation at at least two predetermined wavelengths and where the detector measures the intensity of the fluorescent emission of the sensing compound in the probe in response to each of the two excitation wavelengths independently.

4. The optical chemical sensor system in accordance with Claim 2, where the sensing compound is pyranthrene.

5. The optical chemical sensor system in accordance with Claim 4 where pyranthrene is immobilized in the matrix by being covalently linked to the matrix.

6. The optical chemical sensor system in accordance with Claim 3, where the sensing compound is pyranthrene.

7. The optical chemical sensor system in accordance with Claim 6 where pyranthrene is immobilized in the matrix by being covalently linked to the matrix.

8. An optical chemical sensor system for determining the concentration of an analyte, comprising:

a probe having a polymeric matrix and an optical sensing compound dispersed or immobilized in the matrix, the optical sensing compound being selected from the group consisting of pyranthrene, a derivative of pyranthrene, flavanthrene and a derivative of flavanthrene, and having the property of emitting fluorescence in response to excitation by light, said fluorescence being quenched by the presence of an analyte resulting in a change of fluorescence intensity depending on the concentration of the analyte, said fluorescent intensity being additionally dependent on the excitation wavelength and on the fluorescent emission wavelength, and being affected differently by the analyte at different excitation or emission wavelengths;

light source means for providing excitation light at at least one wavelength;

means optically coupled to the light source means for defining a first optical path for the excitation light from the light source means to the optical sensing compound in the matrix of the probe;

means optically coupled to the optical sensing compound in the matrix

of the probe for defining a second optical path for fluorescent emission light from the optical sensing compound in the matrix of the probe;

detection means optically coupled to said second optical path for measuring emission light intensity at at least two different wavelengths from the optical sensing compound in the matrix of the probe;

computing means coupled to the detection means and receiving input from the detection means for calculating the concentration of the analyte to which the probe is exposed from the measurements of the emission light at at least two wavelengths of fluorescent emission, said calculation utilizing an algorithm based on the spectral response curve of the optical sensing compound.

9. The optical chemical sensor system in accordance with Claim 8 wherein the optical sensing compound is pyranthrene.

10. The optical chemical sensor system in accordance with Claim 9 wherein the matrix is a polysiloxane matrix to which the pyranthrene molecule is covalently linked.

11. The optical chemical sensor system in accordance with Claim 8 adapted for determining the concentration of oxygen.

12. An optical chemical sensor system for determining the concentration of an analyte, comprising:

a probe having a polymeric matrix and an optical sensing compound dispersed or immobilized in the matrix, the optical sensing compound being selected from the group consisting of pyranthrene, a derivative of pyranthrene, flavanthrene and a derivative of flavanthrene, and having the property of emitting fluorescence in response to excitation by light, said fluorescence being quenched by the presence of an analyte resulting in a change of fluorescence intensity depending on the concentration of the analyte, said fluorescent intensity being additionally dependent on the excitation

wavelength and on fluorescent emission wavelength, and being affected differently by the analyte at different excitation and emission wavelengths;

light source means for providing excitation light at at least two wavelengths;

means optically coupled to the light source means for defining a first optical path for the excitation light from the light source means to the optical sensing compound in the matrix of the probe;

means optically coupled to the optical sensing compound in the matrix of the probe for defining a second optical path for fluorescent emission light from the optical sensing compound in the matrix of the probe;

detection means optically coupled to said second optical path for measuring fluorescent emission light from the optical sensing compound in the matrix of the probe at at least one wavelength in response to excitation at at least two wavelengths;

computing means coupled to the detection means and receiving input from the detection means for calculating the concentration of the analyte to which the probe is exposed from the measurements of the fluorescent emission light responsive to the excitation at each of the two wavelengths, said calculation utilizing an algorithm based on the spectral response curve of the optical sensing compound.

13. The optical chemical sensor system in accordance with Claim 12 wherein the optical sensing compound is pyranthrene.

14. The optical chemical sensor system in accordance with Claim 13 wherein the matrix is a polysiloxane matrix to which the pyranthrene molecule is covalently linked.

15. The optical chemical sensor system in accordance with Claim 8 adapted for determining the concentration of oxygen.

16. An improvement in an optical chemical sensor system for

determining the concentration of an analyte, the improvement comprising:

a probe containing an analyte sensor material which comprises a gas permeable composition containing fluorescent analyte sensitive indicator molecules selected from the group consisting of pyranthrene, a derivative of pyranthrene, flavanthrene and a derivative of flavanthrene.

17. The improvement defined in Claim 16, wherein: said analyte sensor material responds with a change in fluorescence intensity depending on the concentration of the analyte, and wherein the system comprises:

a source of excitation light;

means optically coupled to said source for defining a first optical path for the excitation light from said source to said analyte sensor material;

means optically coupled to said analyte sensor material for defining a second optical path for emission light from said analyte sensor material;

a detection system optically coupled to said second optical path for detecting emission light from said analyte sensor material, and

means coupled to said detection system for providing an output reading from said detection system.

18. The improvement defined in Claim 17 wherein: said chemical sensor system excites the analyte sensor material at a first excitation wavelength (EX1) which is responsive with a change in fluorescence intensity depending on the concentration of the analyte, and then at one or more additional excitation wavelengths (EX2) which are responsive with a significantly different change in fluorescence intensity depending on the concentration of the analyte, in which said detection system detects emission light at a wavelength (EM1) from said material for (EX1) and (EX2) independently, and in which said means for providing an output reading from said detection system measures the relative detected emission intensities at

(EM1) from said analyte sensor material.

19. The improvement defined in Claim 17 wherein: said chemical sensor system further excites the analyte sensor material at a first excitation wavelength (E1), and in which said detection system detects emission light from said material at a first emission wavelength (EM1) independently which is responsive with a change in fluorescence intensity depending on the concentration of the analyte, and at one or more additional emission wavelengths (EM 2) which are responsive with significantly different changes in fluorescence intensity depending on the concentration of the analyte, and in which said means for providing an output reading from said detection system measures the relative detected emission intensities EM1 and EM2 from said chemical sensor material.

20. The improvement defined in Claim 16 where the analyte concentration to be determined is selected from the group consisting of oxygen, nitric oxide, sulfur dioxide, and halogens known to quench the fluorescence of polycyclic aromatic hydrocarbons.

21. The improvement defined in Claim 16 where the analyte concentration determined is glucose by means of an enzymatically coupled system.

22. The improvement defined in Claim 16 where the analyte concentration determined is aerodynamic pressure by means of an oxygen concentration coupled system.

23. The improvement defined in Claim 16, wherein: said analyte sensor material responds with a change in fluorescence intensity depending on the concentration of the analyte, and which includes: a source of excitation electric field, means electrically coupled to said source for applying the electric field across said analyte sensor material, means optically coupled to said analyte sensor material for defining an optical path for emission light

from said analyte sensor material, a detection system optically coupled to said optical path for detecting emission light from said analyte sensor material, and means coupled to said detection system for providing an output reading from said detection system.

24. A method of measuring the concentration of an analyte with an optical chemical sensing probe including an optical sensing compound dispersed or immobilized in a matrix included in the probe, the sensing compound being selected from the group consisting of pyranthrene, a derivative of pyranthrene, flavanthrene and a derivative of flavanthrene, the method comprising the steps of:

contacting the sensing compound included in the matrix with a fluid in which the analyte is to be measured;

providing an excitation light to the sensing compound in the matrix of the probe and in contact with the fluid;

measuring the intensity of a fluorescent emission light generated by the sensing compound in the matrix of the probe and in contact with the fluid;

computing the concentration of the analyte in the fluid from the measured fluorescent emission light, said step of computing utilizing an algorithm based on the spectral response curve of the optical sensing compound in the presence of varying concentrations of the analyte.

25. The method in accordance with Claim 24 where

the step of providing an excitation light to the sensing compound in the matrix of the probe and in contact with the fluid comprises providing excitation light at least two different wavelengths;

the step of measuring the intensity of a fluorescent emission light generated by the sensing compound in the matrix of the probe and in contact with the fluid comprises measuring the intensity of said fluorescent emission at a predetermined wavelength in response to each of said two excitations at

the two different excitation wavelengths, and

where said algorithm used in said step of computing is based on the spectral response curve at the two different wavelengths of excitation of the optical sensing compound in the presence of varying concentrations of the analyte.

26. The method in accordance with Claim 25 where the wavelengths of excitation is above 400 nanometers.

27. The method in accordance with Claim 26 where the analyte is selected from the group consisting of oxygen, nitric oxide, sulfur dioxide, and halogens known to quench the fluorescence of polycyclic aromatic hydrocarbons and glucose.

28. The method in accordance with Claim 26 where the analyte is oxygen.

29. The method in accordance with Claim 26 where the sensing compound is pyranthrane covalently linked to the matrix.

30. The method in accordance with Claim 24 where

the step of measuring the intensity of a fluorescent emission light generated by the sensing compound in the matrix of the probe and in contact with the fluid comprises measuring the intensity of said fluorescent emission at at least two different and predetermined wavelengths in response to an excitation at a single wavelength, and

where said algorithm used in said step of computing is based on the spectral curve of the optical sensing compound in the presence of varying concentrations of the analyte at the two different wavelengths of fluorescent emission in response to the excitation at the single wavelength .

31. The method in accordance with Claim 30 where the wavelength of excitation is above 400 nanometers.

32. The method in accordance with Claim 31 where the analyte is

selected from the group consisting of oxygen, nitric oxide, sulfur dioxide, and halogens known to quench the fluorescence of polycyclic aromatic hydrocarbons and glucose.

33. The method in accordance with Claim 32 where the analyte is oxygen.

34. The method in accordance with Claim 31 where the sensing compound is pyranthrane covalently linked to the matrix.

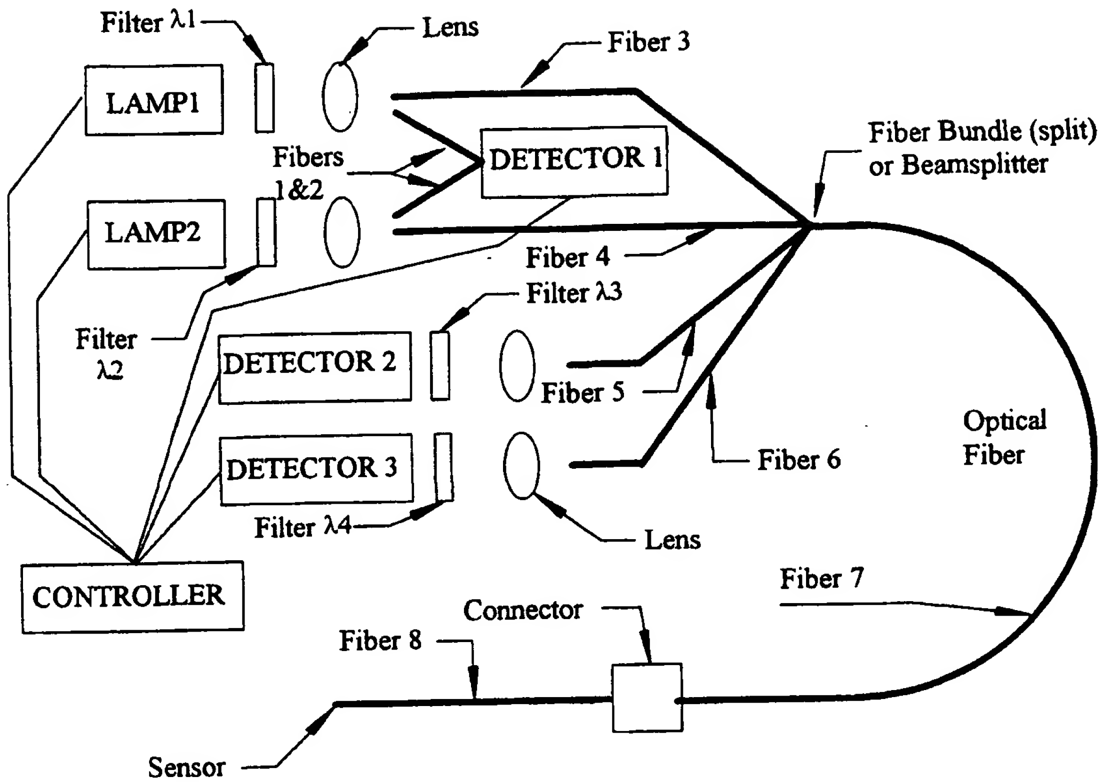

Figure 1

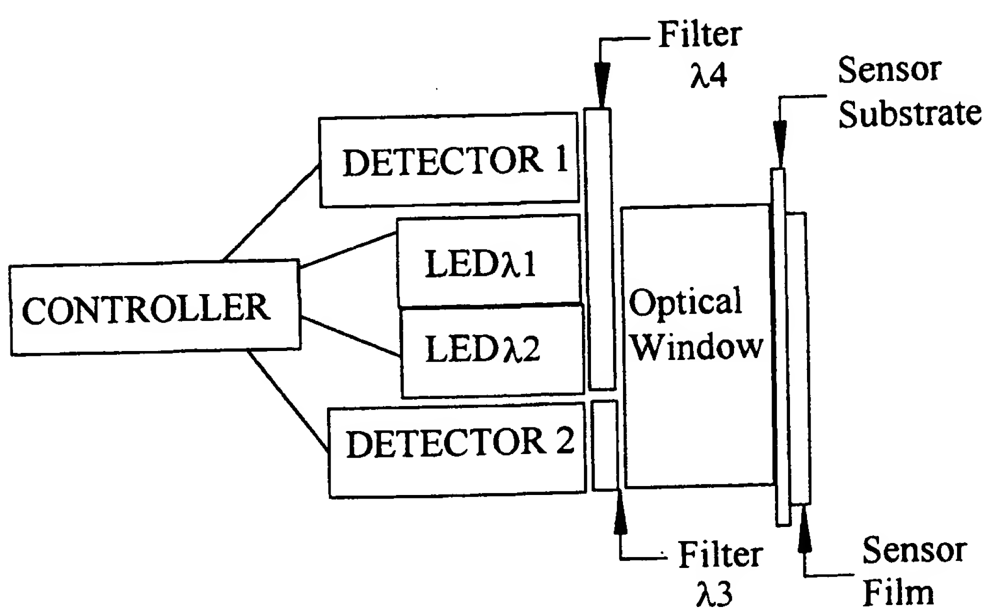

Figure 2

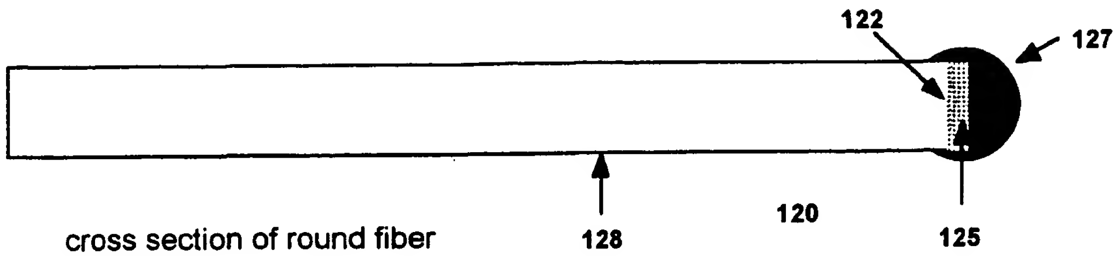

Figure 3

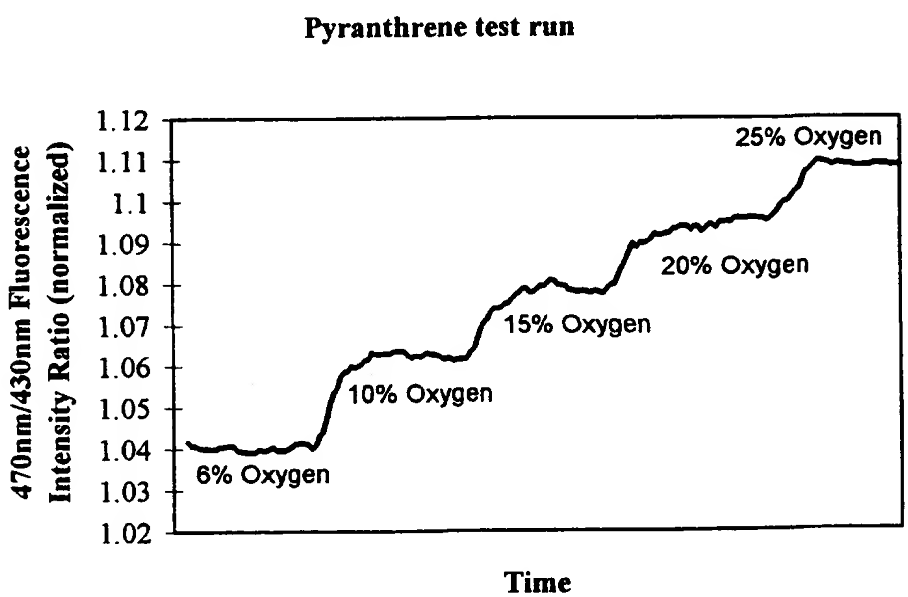

Figure 4

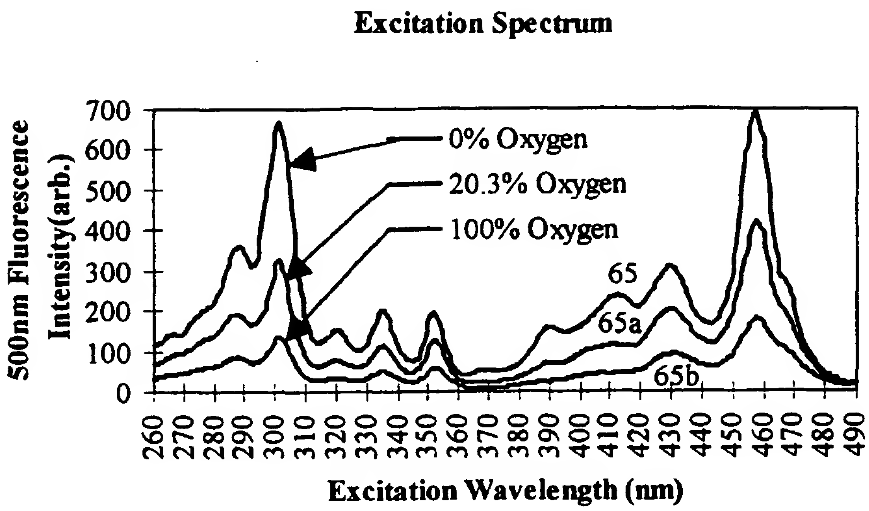

Figure 5

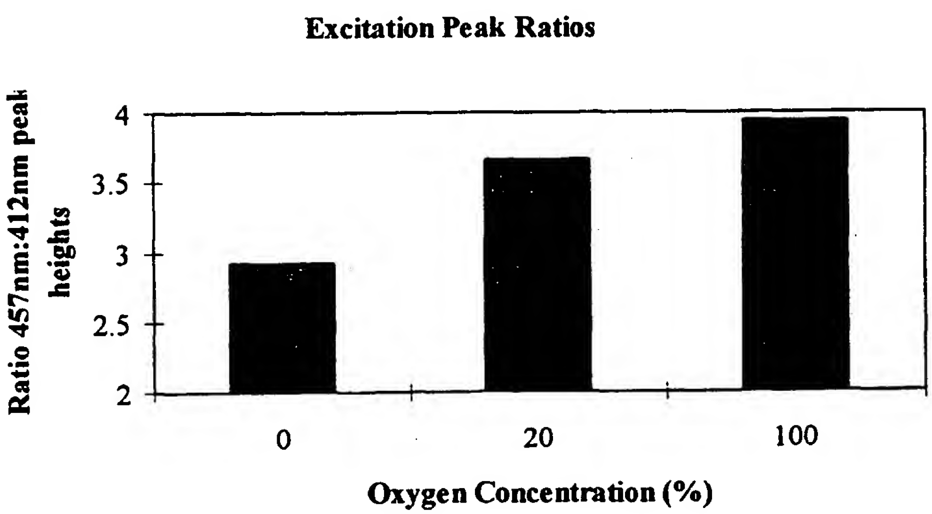

Figure 6

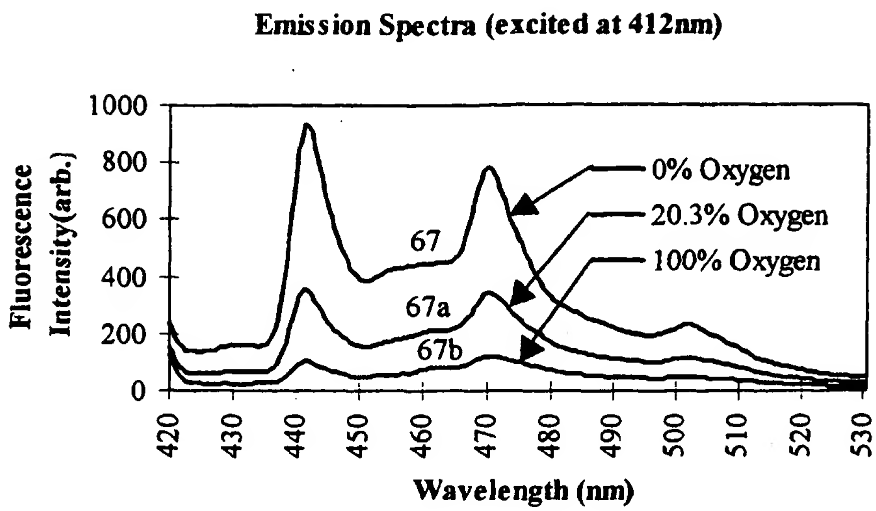

Figure 7

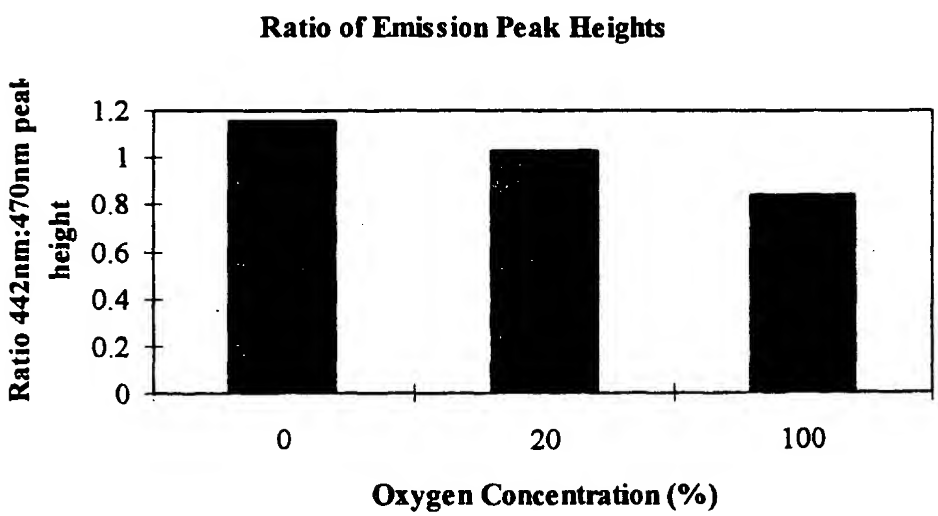

Figure 8

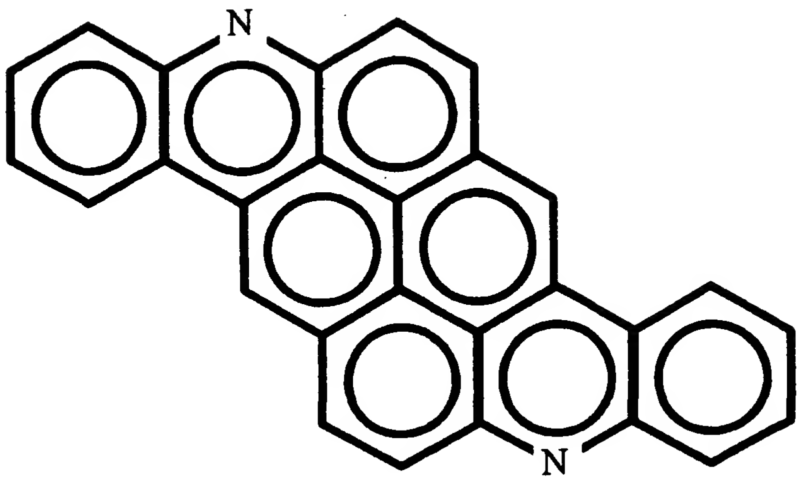

Figure 9

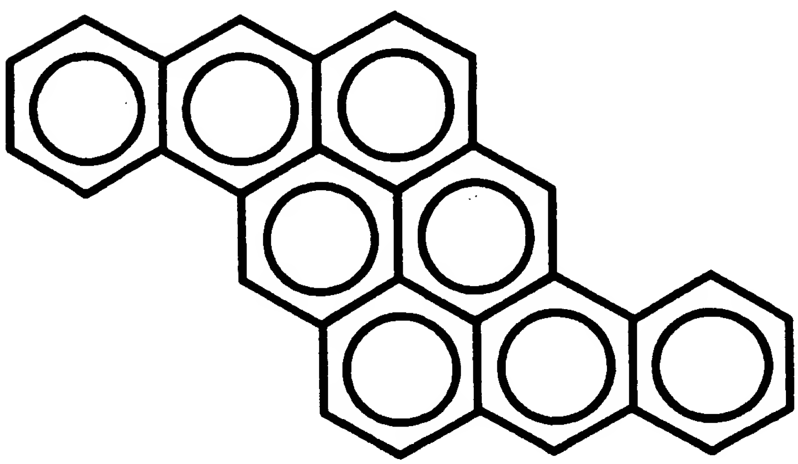

Figure 10

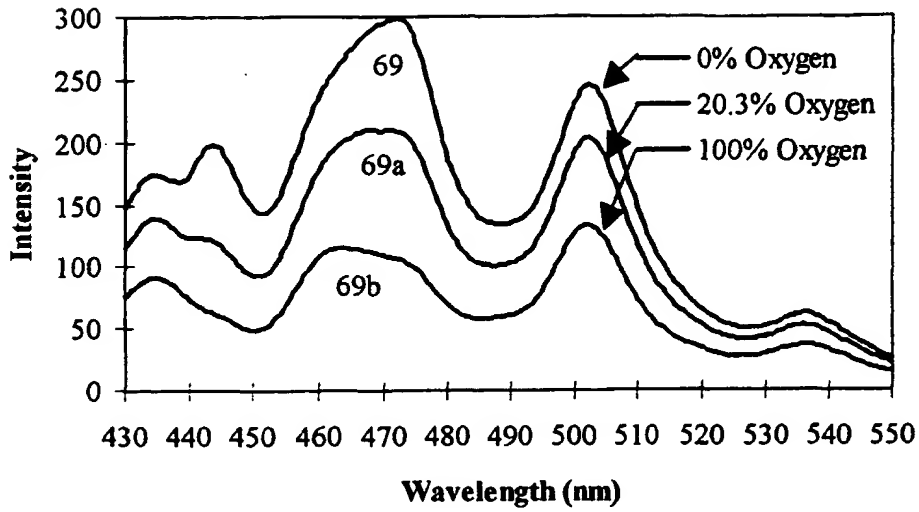
300
250
200
150
100
50
0
Intensity
430 440 450 460 470 480 490 500 510 520 530 540 550
Wavelength (nm)
69
69a
69b
0% Oxygen
20.3% Oxygen
100% Oxygen

Figure 11

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/00800

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 G01N21/64 G01N21/77

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 094 959 A (GOTTLIEB AMOS ET AL) 10 March 1992 (1992-03-10) cited in the application column 3, line 42 -column 4, line 51 claims 1,2; figure 1 ---	1,2,5,7, 8,10,11, 14,15, 17,19, 20,24, 27,28, 30,32,33
A	WO 81 01883 A (ELECTRO NUCLEONICS) 9 July 1981 (1981-07-09) page 30 page 34 claim 1; table 4 ---	1,4,6,8, 9,12,13, 16,24, 29,34

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[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

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"O" document referring to an oral disclosure, use, exhibition or other means

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"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
14 June 2000	21/06/2000

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/00800

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category °	Citation of document, with indication,where appropriate, of the relevant passages	Relevant to claim No.
A	US 5 681 532 A (KANE JAMES ET AL) 28 October 1997 (1997-10-28) cited in the application column 11, line 5 - line 35 claim 1; example 4 -----	1,8,10, 11, 14-16, 20,24, 26-28, 31-33

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INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/00800

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